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Effect of PGPR consortia on nitrate reductase and chlorophyll content of flag leaf of wheat

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Abstract
Present study undertaken to explore the effect of PGPR consortia on Nitrate Reductase and Chlorophyll content of flag leaf of wheat. Nitrate reductase enzyme and chlorophyll 'a', chlorophyll 'b' and total chlorophyll content was measured in flag leaf of wheat. Study resulted, the significantly higher NR activity was recorded in T6 treatment compared to all other treatments. The lowest NR activity was found in control treatment. Statistically, the order of NR activity was T6>T3>T5>T4>T2>T1. Chlorophyll content was significantly higher in T6 which was at par with T4, T3 and T5. The lowest chlorophyll 'a' content was recorded in T1 which was statistically at par with T2. Chlorophyll 'b' content in flag leag of wheat was found statistically highest in T6 and lowest in T2. Total chlorophyll content was again found highest in T6 and was at par with T3, T4 and T5. Data suggested significant effect of PGPR consortia on Nitrate Reductase and Chlorophyll content of flag leaf of wheat.

Keywords: NR activity, chlorophyll, wheat, flag leaf, PGPR consortia

1. Introduction
Increasing production cost due to increasing price of agricultural inputs, stagnating crop yield, low nutrient use efficiency, decline in soil health and pollution of soil and water due to indiscriminate use of chemical fertilizers and agrochemicals are the emerging problems of Indian agriculture. Among the different alternatives, applying microbial inoculants helps to reduce the use of chemical pesticides, and the use of inorganic fertilizers hence represent promising agricultural approaches to sustained soil fertility. Microbes are ubiquitously present; however search of microbes from extreme habitat, diverse ecological niches and possessing novel character is continuous process. Soil microbes and plant are the most important component of soil ecosystem. These two are in constant interaction with each other and has co-existed under similar climatic condition.Therefore, the reduced use of synthetic agrochemicals in crop production and to maintain soil fertility by alternative means is the subject of investigation. It is challenging to continue sustainable agricultural crop production through minimization of harmful effect of fertilization without compromising with yield. Among the different alternatives, application of plant growth promoting rhizobacteria (PGPR) could be a promising substitute. PGPR, in general, are known to have a stimulatory effect on chlorophyll and protein content of leaves and other parameters like growth of roots and shoots Nain *et al*. (2010) [4] showed Stefan *et al*. (2013) [7] and Mahmood *et al*. (2005) [3]. PGPR may be used to enhance plant health and promote plant growth rate without environmental contamination Calvo *et al*. (2014). PGPR of wild plant (Lantana) give the significant result with increasing in shoot length, root length, dry weight in wheat *(triticum* sp.) than PGPR of crop plants (Mustard and maize) Singh *et al*. (2015) [6]. An enhancement of 14-34% in plant biometric parameters and 28-60% in micronutrient content in treatments receiving the combination of AW1 þ AW5 strains, as compared to full dose of fertilizer application. The treatment involving inoculation with AW5 þ AW7 recorded highest values of % P and N, with a two-fold enhancement in phosphorus and 66.7% increase in N content, over full dose application of P and K fertilizers Saharan *et al*. (2012). Therefore, present investigation conducted to study the effect of PGPR consortia on Nitrate Reductase and Chlorophyll content of flag leaf of wheat.

2. Materials and methods
This investigation was carried out during r*abi* season of 2016-17 at Research Farm of ICAR-IISS, Bhopal to study the "Effect of PGPR consortia on Nitrate Reductase and Chlorophyll

content of flag leaf of wheat." The material used and methods adopted during the course of experimentation in the field and laboratory are described under following heads.

2.1 Experimental details

The treatment consisted of:

T_1: Control
T_2: Consortium of PGPR
T_3: Recommended Dose of Fertilizers on Wheat
T_4: Consortium of PGPR + 50%N+ full PK
T_5: Consortium of PGPR + 75% N + full PK
T_6: Consortium of PGPR + RDF

Graded basal dose of N and full dose of P and K fertilizer calculated for 12 kg soil based on recommended dose of fertilizers for the variety (100:60:40), was added to respective treatment pots, except T1 and T2. Pots were irrigated to 60 percent of moisture holding capacity and left for 1 day. Five seeds coated with PGPR isolates were sown in each pot. Control and RDF treatment pot were sown with uninoculated surface sterilized seeds. Per treatment 5 replications were maintained and the pots were kept in net house. For destructive plant sampling and measurement of agronomic parameters, a separate set of same treatment structure was also maintained. Second split dose of N was added at CRI stage and third at flowering stage. Root length; shoot length; root fresh weight and shoot fresh weight was measured by uprooting five plants by random selection from each treatment at eighty day after sowing from the pots meant for destructive sampling. The remaining plants were used for estimation of chlorophyll content and Nitrate reductase activity of flag leaf.

2.2 Chlorophyll estimation from flag leaf of wheat

Estimation of chlorophyll from wheat leaf was done by method of Hiscox and Israelstam (1979) [2]. Fifty milligrams of freshly cut flag leaf was placed in a test tube and 10ml of DMSO was added to it. The tubes were kept at 65°C for 4 hours in a water bath. The extracted pigment was decanted in fresh tube and absorbance of the sample was read at 663nm and 645nm using spectrophotometer and expressed in terms of mg chlorophyll/g fresh weight of leaf.

Concentration of chlorophyll a, b and total chlorophyll was calculated using formula (http://sydney.edu.au/science/biology/warren/docs/spec_chlorophyll.pdf)

Chlorophyll a (mg/ml) = $12.7\times A_{663}$-$2.69A_{645}$
Chlorophyll b (mg/ml) = $22.9\times A_{645}$-$4.68A_{663}$
Total chlorophyll (mg/ml) = $20.2A_{645}$+$8.02A_{663}$

2.3 Nitrate reductase activity measurement in wheat leaf

Nitrate reductase activity in flag leaf of wheat was estimated spectrophotometrically by method of Cazetta *et al*., (2004) [1]. Briefly, 200 mg of freshly cut flag leaf was placed in a test tube placed on an ice bucket. Three ml of ice cold phosphate buffer (pH 7.5), 3ml of ice cold KNO_3 solution (0.2M) and 0.2ml of n-propanol was added to it. The tubes were removed from ice bucket and incubated in dark at 30°C for 1 hour. After one hour the reaction was terminated by placing the tubes in boiling water bath for 5 minutes. The tubes were cooled to room temperature and one ml aliquot from this tube was placed in fresh test tube. One ml sulphanilamide 1% and 1ml NEDD solution (0.025%) was added to it and mixed well. The tubes were left for 20 minutes at room temperature. The intensity of pink colour was read at 540nm using a spectrophotometer. Calculation of Nitrite formed was done by preparing standard curve with different concentration of sodium nitrite. The NR activity was expressed in terms of µmoles of NO_2^-/g fresh weight of leaf/h.

2.4 Statistical analysis

Statistical analyses were carried out through one-way analysis of variance (ANOVA) and the mean of treatments were compared according to Fisher as well as Duncan multiple comparison test. Least significant difference (LSD) was calculated at $p<0.05$ using statistical add on tool package of MS excel (Excelstat).

3. Results and Discussions

3.1 Effect of microbial consortia on Nitrate reductase (NR) and chlorophyll content of flag leaf of wheat

Nitrate reductase enzyme and chlorophyll a, chlorophyll b and total chlorophyll content was measured in flag leaf of wheat. The significantly higher NR activity was recorded in T6 treatment compared to all other treatments. The lowest Nitrate reductase activity was found in control treatment. Statistically, the order of NR activity was T6>T3>T5>T4>T2>T1 (Table1). Chlorophyll content was significantly higher in T6 which was at par with T4, T3 and T5. No significant difference was observed between T4, T3 T5 and T2 in terms of chlorophyll a content. The lowest chlorophyll a content was recorded in T1 which was statistically at par with T2. Chlorophyll b content in flag leaf of wheat was found statistically highest in T6 and lowest in T2. Treatment T1, T5 and T4 were at par to each other and T2 &T3 was statistically at par with each other. Total chlorophyll content was again found highest in T6 and was at par with T3, T4 and T5. Treatment T3, T4, T5, T2 and T1 was statistically at par with each other but T1 and T2 was significantly lower than T6 (Table 2). Moreover, the difference in chlorophyll b and total chlorophyll content in flag leaf of wheat was more subtle between the treatments.

Table 1: Nitrate reductase (NR) activity in flag leaf of wheat as influenced by different levels of N fertilizers and PGPR inoculants.

Treatment	NR activity (µmole NO_2^-/g Fresh wt. of leaf/h)
T1	34.17f
T2	56.24e
T3	100.89b
T4	73.02d
T5	81.26c
T6	111.56a
LSD (0.05)	5.55

Table 2: Chlorophyll content in flag leaf of wheat (mg/g fresh wt. of leaf)

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll
T1	1.70c	0.63ab	2.33b
T2	2.05bc	0.36c	2.41b
T3	2.41ab	0.43bc	2.84ab
T4	2.30ab	0.51abc	2.81ab
T5	2.22ab	0.56abc	2.78ab
T6	2.61a	0.69a	3.30a
LSD (0.05)	0.39	0.26	0.59

Reduction in yield at reduced N dose compared to RDF might be due to insufficient availability of major plant nutrient N which might have interfered with availability of other nutrients as well. This result can be supported by reduction in NR activity (Fig. 1) and total chlorophyll content (Fig.2) at reduced dose of nitrogen. Nitrate reductase (NR) and glutamine synthetase (GS) are the key enzymes of N

metabolism and are also involved in carbohydrate metabolism. The nitrate reductase activity is positively correlated with available nitrogen in soil and influences grain yield and protein content of grain (Xu and YU, 2006) [8]. The pot culture study overall revealed improvement in growth and yield of wheat with inoculation of PGPR at recommended dose of fertilizers. Reduction in yield was recorded with limited supply of nitrogen but maximum benefit of PGPR inoculant was observed at lower dose of N fertilizers.

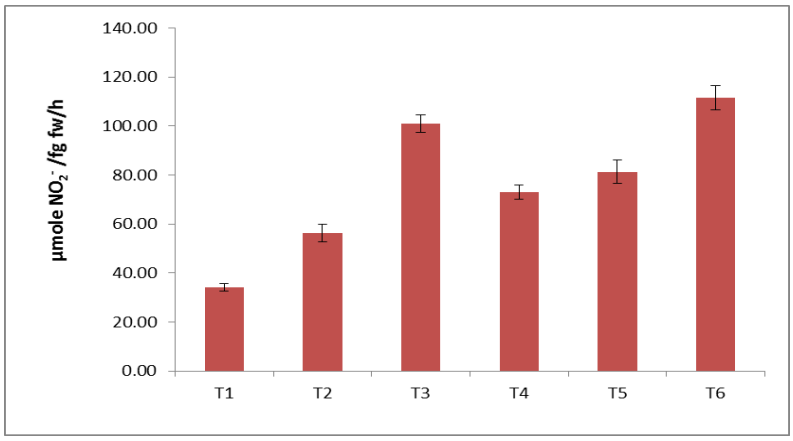

Fig 1: Nitrate Reductase Activity of wheat leaf

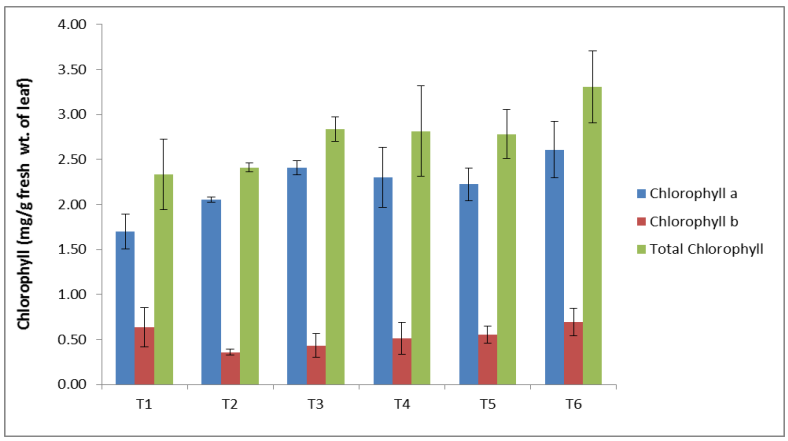

Fig 2: Chlorophyll content (mg/g fresh wt. of leaf)

4. Conclusion

The significantly higher NR activity was recorded in T6 treatment compared to all other treatments. The lowest NR activity was found in control treatment. Statistically, the order of NR activity was T6>T3>T5>T4>T2>T1.Chlorophyll content was significantly higher in T6 which was at par with T4, T3 and T5. The lowest chlorophyll 'a' content was recorded in T1 which was statistically at par with T2. Chlorophyll 'b' content in flag leaf of wheat was found statistically highest in T6 and lowest in T2. Total chlorophyll content was again found highest in T6 and was at par with T3, T4 and T5.

5. Acknowledgement

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